

Frost Hardiness Testing: An Ontario Update

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In the early 1980s large losses of outdoor-overwintered conifer container seedlings were not uncommon in Ontario (Colombo 1982). These losses occurred because seedlings were placed outside in the fall while actively growing and before they were adequately hardened off. Even mild frosts could cause damage and in some cases entire crops were lost (Figure 1). To address these losses, frost hardiness testing methods were developed to provide nursery managers a tool by which to determine when their trees are hardy enough to withstand fall frosts (Colombo et al. 1984).

In the late 1980s overwinter frozen storage of container seedlings became operational practice in Ontario (Odlum 1992). Even though frozen storage temperatures are relatively mild (e.g., -2 to -4°C), nursery stock requires a high level of frost hardiness to stay healthy during storage. Thus, before being stored, it is important that stock intended for overwinter frozen storage be tested for frost hardiness. This note summarizes the method of frost hardiness testing used in Ontario and explains how to interpret the results and apply them to improve overwintering success.

Hardening Seedlings

Seedlings must enter dormancy to become adequately frost hardy for overwintering. In both pine and spruce, dormancy begins with the initiation of terminal buds on shoots. This is usually caused by shortening daylengths, either naturally or artificially. A sequence of warm short days followed by cool short days will

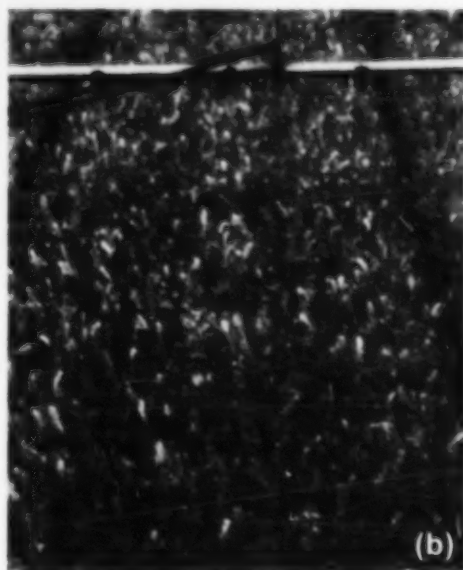
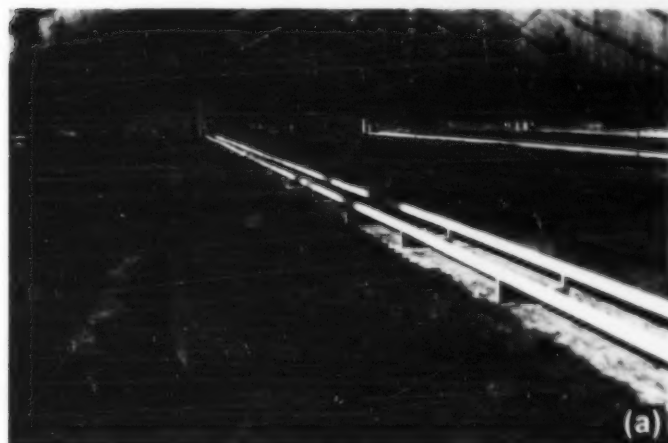


Figure 1. Shoot freezing damage to inadequately hardened black spruce seedlings. (a) Different hardiness levels of stocklots from one nursery reflected in varying amounts of damage (from front to rear). (b) Variation in shoot freezing damage due to hardiness differences among seedlings in a single crop.

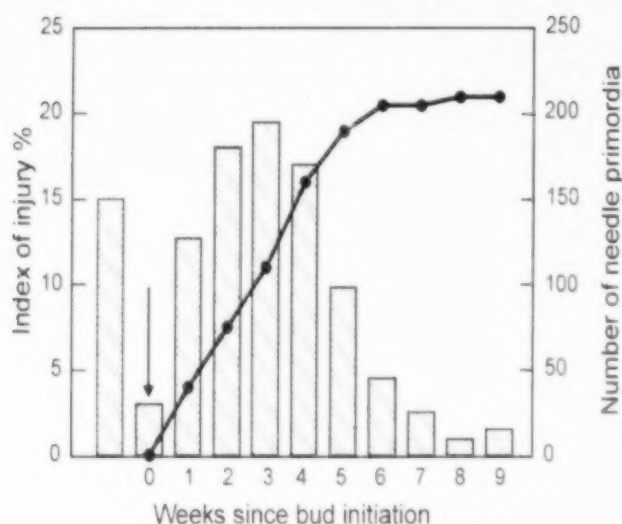


Figure 2. Typical cold hardiness development (bars) and bud size (needle primordia in terminal buds) in a black spruce container seedling crop. Low index of injury indicates high frost hardiness. The arrow indicates time of terminal bud initiation in the crop.

give the best seedling quality results for both overwintering and for planting the following summer. Seedlings hardened in this way will have larger buds, greater diameters, and lower shoot/root and height/diameter ratios (Colombo 1997). It normally takes four to six weeks from the time terminal buds are initiated until seedlings are hardy to -15°C (Figure 2). Exposing trees to cooler temperatures during hardening can shorten this period, but with potentially negative side effects if temperatures are too cold shortly after buds form.

Description of tests

Frost hardiness tests are done using shoot tips to determine readiness for overwintering outdoors or in frozen storage. Shoot tips are collected, subjected to a controlled freezing test, and the resulting damage assessed using the *electrolyte leakage* method.

When freezing damages tissues, membranes around the cells rupture, allowing the cell's contents to leak out. Cell contents conduct electricity. To detect damage, shoots are placed in water, into which the cell contents flow. By comparing the conductivity of the solution with frozen tips to that with non-frozen tips, the amount of freezing damage can be quantified.

The type of test conducted depends on how quickly results are needed. The standard method requires at least three days for results. A quick test has been developed that provides results one day after freezing. Although both tests use measurements of freeze-induced electrolyte leakage from the shoots, the sampling methods differ. Because time is often a limiting factor, the quick test is more common even though it initially requires more shoot tips.

Standard test

The standard test requires 45 shoots for spruce or 32 shoots for pine (Table 1). Shoot tips are divided into groups of equal size and placed in sample jars. Distilled water is immediately added to one jar, which becomes the *control*. It is left to sit at room temperature for 18 to 24 hours after which the electrical conductivity of the solution is measured. The remaining groups are frozen at a rate of 5°C per hour to -15°C (or -40°C if only one test is being done—see below). Once thawed, distilled water is added to the sample jars. After 18 to 24 hours, electrical conductivity of the solution is measured. Following the initial reading, the control and freezer samples are killed by placing them in an oven at 80°C or more for 6 hours. After another 18 to 24 hours, conductivity readings are repeated. Damage in the frozen samples is

compared to that in the control (which theoretically should not be damaged) and heated samples (which should be 100% damaged). Damage is then expressed as an *Index of Injury* where higher conductivity means more damage was incurred and therefore seedlings are less frost hardy (Colombo et al. 1984).

Quick test

To reduce the time required for the frost hardiness test, a separate set of shoot tips is collected to provide the kill sample for each stocklot. This increases the number of shoots required per stocklot for the initial test (Table 1), but only one set of kill values is needed for each stocklot. Therefore, when testing the same stocklot in consecutive weeks, the kill values measured on the first test date are reused.

For this test, shoots are divided into groups of equal weight. As in the standard test, there is one control and two freezer samples per stocklot, but in the first week of testing the remaining shoot tips are divided into a number of kill samples. This allows the kill portion of the test to be carried out while the remaining samples are being frozen (whereas in the standard test, the kill step is not done until after the samples are measured for post-freezing conductivity). Calculations and interpretations of results are the same for both the standard and quick tests.

Sampling methods

The number of shoot tips needed per sample is summarized in Table 1. Shoot tips should be cut 2-3 cm long (from the top of the bud) and collected randomly from across the stocklot, choosing seedlings that appear most succulent as these are likely to be the least hardy. If these seedlings are frost hardy, the rest of the crop will likely be hardy as well. For more consistent results, the same person should collect the samples each week.

Samples should be collected as near as possible to the time they are to be shipped to the laboratory for testing. The shoot tips should be wrapped in damp paper towel and placed in a plastic bag labelled with information such as nursery name, species, seed zone, date collected, and location (e.g., greenhouse or shade area number). The bag(s) of shoot tips should be put in a solid container or padded envelope for shipping.

Trees from different greenhouses, holding areas, seed sources, or other sources of variation in a nursery may not become frost hardy at the same time (Figure 1). Even within a greenhouse and single seed source there may be obvious differences that require separate frost hardiness testing.

Table 1. Number of shoot tips required for frost hardiness testing by test type and crop species.

	First week	Subsequent weeks
Standard test	35 pine / 48 spruce	35 pine / 48 spruce
Quick test	80 pine / 140 spruce	35 pine / 48 spruce

Overwintering tested stock

Stock overwintered outdoors

Nearly two decades of frost hardiness testing to guide the overwintering of container stock has been done in Ontario (Colombo 1997). Similar testing has been conducted for over 10 years in Manitoba and the Maritime provinces. Experience has shown that seedlings that have passed two consecutive -15°C frost hardiness tests (index of injury 5% or less) can usually be safely moved outside as late as the end of October. This is based on temperatures outside not being colder than about -20°C . It also presumes that the trees received a period of cold temperatures (e.g., around 5°C) in the greenhouse to increase root hardiness. Before stock is moved outside (or placed in frozen storage), stems should be checked to ensure they are lignified (generally indicated by being brown all the way to the end of the stem, not green) and long white roots should not be visible.

If moved outside late in October the risk of damage is initially high, even if shoots have passed the frost hardiness test. For example, while shoots may be hardy to temperatures below -15°C , roots are likely to be significantly less cold hardy, so tray edges need to be insulated to minimize root freezing. Damage can also occur the moment trees are moved outside if they undergo rapid freezing (rapid freezing is almost always lethal even if the minimum temperature reached is the same as that reached during slow freezing). Generally, when trees are being moved outdoors they need to be protected from wind if the difference in temperature from the greenhouse to outside is more than about 5°C .

Frozen stored stock

Even though frozen storage temperatures are not very cold, seedlings require a high level of frost hardiness to survive the period of prolonged dark, frozen conditions. For this reason, stock is considered ready for frozen storage three weeks after it has passed two consecutive -15°C freezing tests (index of injury in both tests 5% or less). The same level of hardiness can be determined by passing one freezing test at -40°C (index of injury 5% or less). Stock placed in storage before passing one of these criteria may be damaged in storage and not perform adequately after planting. In addition, protective measures, such as the use of fungicide to prevent the spread of *Botrytis* and plastic-lined boxes to avoid desiccation, help to ensure the success of overwinter frozen storage. Frozen stored stock is less subject to environmental fluctuations than stock that is overwintered outdoors, but care is nevertheless needed to avoid warm temperatures during freezing and thawing (Odlum et al. 2001, Paterson et al. 2001).

Conclusions

Aside from stresses that seedlings may be exposed to after planting, overwintering is the time nursery stock is most likely to be damaged. Frost hardiness testing is a tool that helps nursery managers and foresters to determine whether their trees are ready for overwintering. As with other decisions about nursery crops, the information must be applied correctly for full benefit. Inadequate or incorrect sampling or failing to wait until crops have passed frost hardiness test standards before freezer-storing or moving stock outdoors can result in seedling damage and/or mortality.

More information about frost hardiness testing and proper overwinter seedling storage procedures is available from the Ontario Forest Research Institute.

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